THE MORPHOGENESIS OF THE STIGMATA AND STOMATA OCCURRING IN PERITONEAL AND VASCULAR ENDOTHELIUM

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WITH TWO PLATES

PREFATORY NOTE

Although a great many papers have been written which, either directly or indirectly, involve a discussion of the stigmata and stomata, their significance has never been definitely determined. The majority of the discussions have to do, not with these structures themselves, but with their influence and importance in certain physiologic and pathologic processes. Their existence as real morphologic elements has been assumed and then their function or office argued on *a priori* grounds. This method of study is always uncertain and doubly so when morphologic problems are under discussion.

The study of which this paper is an excerpt was begun nearly ten years ago, when the writer was still in his undergraduate course. It at first involved surgical problems only, but it soon became evident that anatomic knowledge was inadequate for successful study of surgical and pathologic problems. A review of the literature gave but little aid and less encouragement, for it was apparent that the history of our knowledge of the structure of the peritoneal endothelium[1] is coincident with that of the action of the nitrate of silver upon this structure. A reconsideration of the problem with the aid of modern technical methods seemed imperative. In order to meet this requirement the writer has tried to bring to bear on this problem the fruits of modern perfected histologic technic.

[1] Waldeyer advises (*Arch. f. mikroskop. Anat.*, Bd. 57, Heft I, S. 4, 1900) that the term "endothelium" be retained for the cells lining the blood and lymph vessels and the chambers of the eye only. In this paper, as a matter of convenience, the advice of this eminent authority will be disregarded and the term endothelium will be used according to the original suggestion of His.

The present communication has to do with the so-called stigmata and stomata only (as occurring between endothelial cells) and discussion will be confined to these, with casual reference to other facts determined by these studies when they serve to illustrate the points under discussion.

Most of the work was carried on far removed from most of the advantages desirable for this kind of work. This misfortune has been especially felt in reviewing the literature, and the writer regrets to state that he has been unable to confirm, at the last moment, his citations. Since some of the papers were read years ago, only notes were available at this time, and many errors will probably have been committed.

It is pleasing to state, however, that a part of the work was done in the laboratory of Dr. H. Virchow, Professor in Berlin. It is with pleasure that the writer takes this opportunity of expressing his appreciation of the many kindnesses received while doing this part of the work. The obligation presents a wider range than can be mentioned and represents every favor that a teacher can bestow upon a pupil.

The writer is also indebted to Dr. Kopsch for many suggestions in technic.[2]

CITATION OF LITERATURE

As is well known, it was Coccius and Flinzer (1) who first used the nitrate of silver to demonstrate the cell outlines of Descimet's membrane. This method was perfected by His (2). The method was still further developed by v. Recklinghausen (3) and used by him in his study of the lymphatic system. It was while using this method, in the study of the peritoneum, that Oedmasson (4) first noticed the occurrence of small dots and rings in the intercellular lines. The discovery was accidental, for, as the title of his paper indicates, he was concerned only with a review of v. Recklinghausen's work. Oedmasson himself hardly regarded the discovery seriously, but was rather inclined to believe that the rings were real openings because of their regularity. The stigmata he regarded as deposits of silver albuminate because of their irregular size and form. Not until after the same structures were observed by His (5)

2 Professors Sayer and Bailey, respectively heads of the departments of pharmacy and chemistry in the University of Kansas, have rendered aid in the consideration of chemical problems. To them also the writer expresses appreciation.

in the lymph vessels was Oedmasson's discovery regarded as of importance. From then on the belief in their entity gradually became general. A number of other papers were written about this time, the most important of which were those of Ludwig and Schweigger-Seidel (6), Dybkowsky (7), and Schweigger-Seidel and Dogiel (8), none of which added greatly to our knowledge of the subject in advance of that presented in previous publications.

The highest interest in the stigmata and stomata was not reached, however, until Cohnheim (9) described them in the blood-vessels and used them as important elements in his theory of inflammation.[3] It is to this fact, no doubt, that these structures owe much of their prominence, for after their acceptance by Cohnheim their existence was almost universally regarded as proven, though no additional evidence had been adduced to strengthen the position.

That the existence of these structures as morphologic elements was never established upon a sound histologic basis may be gathered from the fact that very soon after they were first described there were not wanting investigators who regarded them as artificial products, due to the reagent used. Among the earlier writers to express their opposition may be mentioned Auerbach (10), Afanassiew (11), Foa (12), Klein (13), and Schweigger-Seidel (l. c.). The last two authors regarded them as real openings, but declared against the function ascribed to them on the ground that no opening could be demonstrated in the basement membrane (membrana limitans) without which the stomata would necessarily be functionally useless. Later on Klein seems to have changed his view somewhat (14 and 15), ascribing more importance to the stomata, even going so far as to class them in two groups, stomata vera and pseudo-stomata. In the former group he reckons only those occurring in the center of groups of radiating cells. To the list of opponents must be reckoned Tourneaux (16). He is very emphatic in his opposition, declaring without qualification that they are artefacts. He based his argument on the physiologic fact that starch and carmine granules are not absorbed when an emulsion of them is introduced into the free peritoneal cavity.

These papers were written about the time Cohnheim announced his theory of inflammation. So brilliant were his discoveries, and

[3] He accepted the views as expressed by Stricker and Federn as correct (*Wien. Acad. Sitzungsber. math.-nat. wis. Cl.*, Bd. 53.

so striking his explanation of the phenomena he observed, that the arguments of the opponents of the theory of the existence of special openings between cells were futile. It seemed so clear that the leucocytes, and above all the red corpuscles, in order to escape from the blood-vessels, required the existence of openings that would allow of their escape. The discovery of Oedmasson was eagerly seized upon as offering the needed explanation. Here it seems necessity added one to her progeny: already the mother of invention, she now became the mother of discovery also.

Arnold (17, 18, and 19), by his careful researches, aided greatly in strengthening the position of Cohnheim's theory and more particularly the idea of the passage of the blood-cells through preformed openings. It was he who first observed the blood-cells actually to pass through the preformed openings, thus adding demonstration to theory. Arnold (20) presents a cut showing a leucocyte in the act of passing through one of these openings. This has not been observed by any other worker. It was this striking demonstration of Arnold's more than any other factor, apparently, that despaired the opponents of the theory.

Notwithstanding the reputation of the author, however, doubts began to arise as to the correctness of the observation, mostly, no doubt, on account of the extreme difficulty of excluding error in determining the exact point in the vessel wall at which the blood-cell made its escape. So uncertain is this method of determining this point that investigators may well despair of direct disproof.

Another paper that has had much weight in influencing opinion is that of Muscatello (21), who approached the subject largely from the physiologic side. He ascribes to the stomata the office of absorption, but declares that they exist only on the centrum tendenium of the diaphragm.

Ranvier (22) advanced a most convincing argument against the stomata when he showed that their occurrence could be much reduced in number by first rinsing the surface to be treated with silver solution with distilled water. He also added the additional important argument that their irregular distribution counted much against their functional importance.

Whatever opinion one may hold as to the cause of it, the fact remains that the stomata no longer play the part they formerly did in the explanation of physiologic and pathologic processes. To such

an extent is this true that the literature of the last decade, of physiology and pathology, may be searched without encountering a single reference to them. This fact might be interpreted to mean that these structures have been abandoned and that the subject no longer offers a fruitful field for investigation.

That this neglect has not been altogether universal is shown by several recent papers dealing with the histology of the peritoneal endothelium. The most elaborate and complete of these is by Kolossow (23), in which the stomata are restored to their former position. It may be stated briefly in passing that Kolossow makes a modification in that he regards the endothelial cell as being formed of two parts or layers, a granular part containing the nucleus, and a superficial homogeneous part which he calls the cover plate (*Deckplatte*). It is between these latter that he regards the stomata as existing, and not beween the cells proper.

More recently a paper has appeared by Ussow (24) in which a similar position is taken. He believes that ordinarily they do not exist but are brought about by the contraction of the cell protoplasm. In the short abstract at my disposal no reasons are given for this conclusion.

In practical works the stomata still play a more important rôle than among scientific workers. This is well shown by an excellent practical treatise on the peritoneum (27) in which the stomata have ascribed to them their former importance.

The other side of the question has recently been strengthened by a very clear paper by Meyer (25). He concludes that the stomata are not real openings. He arrives at this conclusion because of their irregular distribution and because of their irregular size and shape. He believes, however, that they may be artificially produced by mechanical means, though without ascribing to this fact any specific importance.

Rawitz[4] takes a delightful middle ground by saying that if stomata do not exist there are at least "soft places."

Nearly all investigators have used the same method. This consists, briefly, in treating bits of excised tissue to a solution of silver nitrate and then exposing them to the sun until they become brown. Kolossow (26) alone forms an exception in that he used a solution of osmic acid and then "developed" in a solution of tannin. By

[4]Grundiss der Histologie. Berlin, 1894.

this method neither stigmata nor stomata are produced, and his views concerning them are dependent upon the use of silver used according to the usual method.

The review of the literature shows that but one method has been used in demonstrating the stigmata and stomata, which suggests that inquiry should be directed quite as much to a criticism of the technic as to the real problem itself.

Whether approached by a direct or by an indirect method, the real problem is, Are the stigmata and stomata preformed openings between endothelial cells? If a negative answer is the truth, the question becomes, What is the nature of the so-called stigmata and stomata? An answer to the second question is desirable, not only on account of its interest because of the discussions these structures have occasioned, but because the disposal of them can not be regarded as final until they have been proven of extraneous origin by direct analysis.

FORMS OF STIGMATA AND STOMATA

The stigmata have never occasioned any considerable amount of discussion, and they may be considered along with their more prominent kin. In order to facilitate the study of the stomata, it is desirable to divide the various structures that have from time to time been included in this category into two classes. In the one class may be considered the ordinary form, which may be seen in nearly every cut of the peritoneal endothelium that has been published. In the other group may most conveniently be considered a great variety of different structures that have been regarded as stomata by some observers, or structures incidentally mentioned but which throw some light on the formation of the more common form.

The ordinary stigmata and stomata are such familiar structures that a definition is hardly needed, and a satisfactory one is difficult to formulate because it is necessary to regard the opinion of so many different observers. It may, however, in general be stated that the stigmata are small brownish black points, varying in size from a fraction of a micron to ten microns, usually irregular spheroids, but often angular, occasionally elongated ovoids, occurring at irregular intervals in the course of the intercellular lines between the endothelial cells, when a serous membrane is treated with silver nitrate and exposed to the sun. By stomata are meant the brownish

rings, ovoids, or ellipses, occurring under similar conditions as mentioned above, situated anywhere in the course of the intercellular line, but occurring most typically at the point of juncture of three or more cells.

Stigmata and stomata have not been noted, so far as the writer's knowledge goes, in any other structure than a serous membrane, nor in this structure when treated by any other reagent than nitrate of silver. They may be regarded, then, as the product of two factors; nitrate of silver and a fresh serous membrane. The formation of these structures was believed to be due to the action of the silver nitrate upon the semi-fluid intercellular cement substance, forming an albuminate of silver. Why a border of the albuminate occurs about the stomata has been explained by the fact that the stomata are formed by a stretching of the membrane, and when the border of the cells retract a part of the cement substance remains adherent which, when acted upon by the silver, forms the stomata. In order to account for the fact that the cells separate only at certain points it was necessary to assume the intercellular substance to be stronger at some places than at others.

The relation of the stigmata to the stomata was at first thought by Arnold to be direct, in that he assumed the former to be but early stages of the latter. In his later publications he described them as being but local broadenings of the cement substance—an unskilful distribution of the adhesive, as it were. Most other observers share this view, while a few disregard them entirely.

The exact method of formation of the stomata, as has been stated, has caused considerable controversy. Some regard them as preformed, others as due to the passage of leucocytes, but the majority regard them as due to the stretching of the membrane. The cells were believed not to be quite big enough to cover the entire surface when the membrana limitans is put on the stretch. The writer has made a long series of experiments to determine this point. The result was invariably that, when the membrane was sufficiently stretched to produce a solution of continuity of the cells, they did not separate in the intercellular lines but tore across the cell in the great majority of cases, the direction of separation depending on the line of rupture of the membrana limitans. The cells do not separate so long as the membrana limitans is intact. Even if by chance the cells do separate in the intercellular line it does not occur

at certain points but occurs in a straight line. Kolossow worked out this point and attempted a direct demonstration of the theory. He did this by stretching a serous membrane across the end of a glass tube and then bringing about varying degrees of distention. He found that the stigmata and stomata increased in number as the distention was increased, and in direct proportion.

The theory of their formation by the leucocytes was abandoned on the ground that when a vein is tied in which diapedesis has begun it ceases. If due to the activity of the leucocytes the process (of stomata formation) would go on.[5]

If the distention theory of formation were true it would seem that a great variety of normal stretchings of the serous membranes would produce them and be followed by the usual consequences. But, as is well known, the distentions may be greater under some normal conditions than under some pathologic ones in which diapedesis does occur, without this process taking place.

If, in the study of the genesis of the stigmata and stomata, the classic method of treatment, with silver nitrate and exposure to light, be followed, the basis of the whole controversy can be readily observed, for all the different pictures described by the various investigators may be made out. In reviewing the results the most striking observation is that of Ranvier, already referred to, namely, that the number of stomata may be lessened, and for large areas prevented, by a preliminary rinsing with distilled water. So striking is this fact that the probability at once suggests itself that these structures are due to some factor that can be removed with distilled water. That actual openings could be thus removed of course no one would for a moment argue. That large areas may thus be obtained free from stomata is a fact various observers have vouched for. Why the cells in such large areas should occur without the ability to perform the same functions as identical cells in other localities is a question Ranvier asked years ago and which still remains unanswered. Observers are likewise agreed that exposure to the sun increases the number of both stigmata and stomata. The same thing is true if the membrane is covered with an excess of lymph.[6]

[5] Muscatello still adheres to this view, l. c., p. 345.

[6] Oedmasson (l. c., p. 362) correctly states that the lines are broader if the strength of the solution is stronger, or allowed to act longer. He also correctly observes that it is due to the action of the silver on the cement substance and not due to its action on the edge of the cell, for the lines may be penciled out. He might have added that this in no wise influences the distance between the edges of the cells, for the increase in width is due to deposits on top of the cells (see figs. 3 and 4, Plate XIII).

The problem presenting itself for solution is obviously, from the foregoing, to determine how many stigmata and stomata would remain if all the factors known to cause them were excluded entirely, and the factors known to increase them were reduced to a minimum. But with the methods ordinarily employed carried out with the greatest care, one finds himself where he must accept the striking observation of Toldt (28), that "they occur in spite of every precaution, but regarding their significance we are entirely ignorant."

It seems evident from this quotation that advance can be secured only through new methods or by improvements on the old ones.

The effect of light is a factor that has never been systematically studied. Handling the tissues in an entirely dark room is difficult and uncertain. An attempt to solve this problem led the writer to develop a new method of using silver. Knowing that Ranvier had injected silver solutions into the adipose tissue in recently killed dogs, in order to demonstrate the fat globules, it required but a step farther to attempt to secure the reaction *in vivo*. The results obtained were gratifying in the extreme, for nothing is simpler than to inject a dilute solution of silver nitrate into the free peritoneal cavity of an animal. The effect of light may thus be determined with a certainty. The anterior abdominal wall of a mouse so treated may be removed in the dark by the aid of the sense of touch and placed upon a slide and the microscope so arranged that the first ray of light that strikes the specimen may be met by the eye of the observer over the microscope. The intercellular lines are seen to be present, thinner and more regular than after the usual method, and stigmata and stomata are not to be found. How much the result may be due to the action of the reagent on living tissue and how much on the exclusion of light must be determined by comparing the results obtained by injecting the solution into the abdomen of a dead animal. For the purpose of the present discussion it makes no difference. The important fact remains: the occurrence of both stigmata and stomata can be entirely prevented. In some animals so treated the usual structures occurred. This is most likely to occur in frogs, especially when the solution is too strong or allowed to act too long. In order to test this method the following animals were injected successively: five new-born mice, six grown but young mice, one pregnant mouse, four new-born rabbits, one old buck

rabbit, and three kittens six weeks old. In none of them was a single stigma or stoma to be found. In this study it was necessary to remove the parietal peritoneum from the abdominal wall (except in case of the mice and new-born rabbits, in which the abdominal wall was sufficiently transparent to admit of examination *in toto*); the mesentery was also carefully removed, the whole intestinal canal cut open along the mesentric border, after the removal of the mesentery, and examined over its entire extent, and finally the diaphragm and special ligaments were removed as much as possible entire. Of course some nooks and corners escape observation, but with care they may be reduced to a minimum. This series of animals, seemingly in perfect health, were able to so live without a single stigma or stoma—so much is certain.

The injection method enabled the writer to test the distention theory already mentioned as being held by a goodly number of observers. By injecting a large amount of a dilute solution into the free peritoneal cavity any degree of distention may be produced. This would serve only, of course, if the endothelium of the abdominal wall were the object of study. If it is desired to study the endothelium covering the intestinal canal a small amount may be injected into the abdominal cavity and the intestine then distended with air. Mice, because of their cheapness and convenient size, were most used in these experiments. For the sake of comparison several other animals were injected, namely, several rabbits, rats, and a cat. Larger animals, for obvious reasons, were not experimented upon. After many trials under the most varied conditions, it can be stated that stomata do not occur under these conditions. This method of testing the distention theory would seem to be an ideal one, for the tissues are uninjured in any way, and there is no escape of blood or lymph to obscure the results.

On account of the influence the cell form was believed to have on the production of the stomata, it will be proper to consider the effect of simple distention on the cell outline. The distention experiments before mentioned show that the cells are large enough to cover the membrana limitans without changing their form to any appreciable extent. When a serous membrane has less surface to cover it forms folds, and the individual cells do not contract. These folds are microscopic, and they have given rise to confusion because various deposits occurring in them have been mistaken for stomata.

Too much stress can not be laid on the importance of experimental study on this point on account of its bearing on the problems of stomata formation.

Another method of determining the effect of distention may be briefly mentioned. An abdomen may be distended with air and the intercellular spaces made apparent by painting the surface of the abdomen with a solid stick of silver nitrate. The cell outlines are brought into view. No influence on the production of stomata can be observed. It may be mentioned in passing that the silver thus applied acts not only through the whole thickness of the abdominal wall, but to a considerable depth beyond. This is, in a sense, a contradiction to the generally accepted opinion that the action of silver is very superficial.

Still another way of demonstrating the absence of openings between cells is as follows: A bit of the abdominal wall is stuck to a slide, endothelial surface down.[7] After firm adhesion had taken place, the membrana limitans with all the other tissue may be removed, leaving the endothelium alone adherent to the slide. It may now be treated with any desired stain, preferably fuchsin. The edges of the cells will be found to lie closely together without the existence of openings. The last method gives some very satisfactory results, though the method is somewhat uncertain.[8]

In the second group may be considered together all those structures that have been regarded as stigmata or stomata which are excluded in the first group. This will include all structures that have been described as such in the literature.

Structures identical with those of the first group have been figured in every conceivable place that silver can penetrate. In many cuts the stigmata may be seen scattered promiscuously over the cells.[9] Stomata likewise may occur in a great variety of places. They may often be seen in the body of the cell forming frequently gyrated figures (see fig. 1, pl. XII). Again the circles may occur in the intercellular line but in which the line continues uninterrupted through it. These must be distinguished from objects occurring

[7] Afanassiew's method (l. c., p. 58) was used.

[8] The writer has for some time been experimenting with iron and tannin for bringing out the cell outlines. By this method, likewise, no stomata appear, nor are the cell outlines affected by stretching.

[9] Meyer very properly asks that, if they are "to be called stigmata in one situation, why not in the other?" l. c. Robinson is the only writer who is willing to accept them in this abnormal situation (The Peritoneum, p. 53. Chicago, 1897).

below the endothelium. These stomata may be seen below the endothelium, between the muscle fibers (fig. 3, pl. XII). These may, instead of forming rings, form solid patches, thus resembling a cell. These were pictured in Oedmasson's original publication, and they may be seen in many productions since that time, but it remained for Meyer to recognize the true conditions. Fig. 10 in his paper presents the appearance very well. These same structures may be seen lying upon the endothelium of the lymph vessels.

A modification of the regular stomata, already alluded to, is insisted on by a number of investigators: it is the occurrence of openings in the center of radiating groups of cells. Nikowlsky laid especial stress on these and pictures several marked examples. A few writers, notably Klein, regard these as the only true stomata. These are certainly striking figures, but it is difficult to understand how these came to be regarded as the true stomata, for it often requires prolonged search before one can be found. Their rarity alone ought to be sufficient to refute the arguments that have been advanced relative to the function ascribed to them. Attempts to study them on cross-section have been fruitless.

The significance of the occurrence of extraneous cells between the regular endothelial cells has been the theme for a great deal of discussion. Broadly speaking, they have been regarded as either leucocytes or young endothelial cells, or again as cells destined to form endothelial cells (*Keimzellen*). No attempt has been made to separate the cells actually occurring between the endothelial cells from those located either above or below them. This is quite necessary since these cells have often been regarded as guards for the stomata, filling them up valve-like, as it were (Ranvier), or, when impregnated with silver, they have been described as stomata.

Much confusion has arisen because no attempt has been made to determine the exact location of the cells. This is beautifully illustrated by a cut Robinson[10] borrows from Stöhr, in which nuclei of cells lying underneath the regular cells are shown. Stöhr[11] regards them as nuclei of connective tissue cells (see fig. 6, pl. XII), and Robinson calls attention to the fact that these have always been regarded as stomata. It is quite imperative that this differentiation be carried much farther. This is often not an easy matter, for even

10 l. c., p. 29.

11 Stöhr, Lehrbuch d. Histologie, 7te Aufl, S. 220.

with modern microscopical appliances it is often difficult to determine whether a particular object lies above or below the endothelial cells. This is more difficult in silver preparations because the silver is thrown down in fine granules, which cause a confusing refraction of the light. When objects are below the intercellular line it is often possible to determine this fact. With this exception it seems advisable to exclude all evidence that can not be proved on cross-section.

Cells above the endothelial cells are but rarely found. The writer for a long time observed cells in this situation, believing them to be endothelial cells, which were contracted from irritation, as described by Ranvier. On cross-sectioning the tissue the endothelial layer was found to be intact. Attempts to determine their relation to inflammatory processes, experimentally produced, have been without result.

The first observer to note the occurrence of small cells between the larger ones was Dybkowsky. Klein advocated the view that they were cells in course of development. Ranvier regarded them as leucocytes filling up the stomata. Virchow[12] has been incorrectly quoted as advocating this view. As a matter of fact the statement was made that "multinuclear cells occur below the epithelial cells of the peritoneum." These words were written before the controversy began and before it was known that leucocytes occurred outside of the blood-vessels; indeed it was in support of the theory that they do so occur that the statement was made. The leucocytes are really the only ones that ought ever to have been mistaken for stigmata or stomata, for, as Tourneau and Hermann pointed out, the oblong nuclei and the bright nucleoli are always sufficient to distinguish the other cells occurring in this locality from them. This discussion could have arisen only from a failure to study them in cross-section.

The exact significance of these cells is not patent to the subject; it is sufficient for the purpose to show that they form a part of the regular cell layer.

It is the cells located below the endothelial cells that have been mistaken for stomata the most frequently, and in consequence are of greater interest in this connection. Many fine examples of this confusion may be seen in the literature, notably in the publication of Oedmasson. Kolossow classes them all as leucocytes. As al-

[12] Gesamte Abhandlungen, S. 167. 1856.

ready mentioned, Stöhr pictures several as connective tissue cells. Both kinds do no doubt occur in this place. There are still others occurring here probably belonging to the clasmatocytes of Ranvier[13] or to those large cells which are now regarded as taking some part in the process of inflammation. It is sufficient to say that they are all located below the endothelial layer.

It is necessary to note that there are fields without nuclei occurring between the endothelial cells. The explanation for this occurrence is not easy. It is quite possible that it is a cell below the regular cells taking its place in the regular layer.

With the silver method alone it is quite impossible to distinguish the various cells from one another, and this problem must be solved by the aid of other stains. With these it may be determined to a certainty that the great variety of cells that have been at some time or other believed to bear some relation to the stomata are entirely independent of the endothelial layer. Their exact office is a problem that remains to be solved. The silver method will not aid in solving it. Indeed it is to the uncertainties of this method that much of the confusion is due, aiding much in making the histology of the peritoneum "the darkest chapter in anatomy."

CHEMISTRY

Now, thoroughly convinced that the stigmata and stomata are spurious products formed by a precipitate of silver, a determination, if possible, of their exact chemical composition seemed very necessary. As is well known, the intercellular lines are supposed to be formed by the action of the silver nitrate upon the albuminous cement substance, forming an albuminate of silver. This assumption has been accepted by all writers. In approaching this subject the writer thought to prepare himself for the investigation by studying the chemical nature of the substance under consideration. He was surprised, after spending some time in a literary search, to find no mention of silver albuminate in the most extensive works on chemistry, in any of its departments. Surprise rose to amazement when one of the ablest of chemists was appealed to for aid and the reply was made that he knew of no such substance.

Here, then, was a virgin field for investigation. Returning to the

[13] "Des Clasmatocytes," *Archives d'Anatomie Microscopique*, Tome III, p. 122.

observation of Ranvier that the occurrence of stomata could be prevented by rinsing the membrane before applying the silver, it was quite natural to remove some of this fluid for examination. Some of the fluid normally covering the free surface of the peritoneum was carefully transferred to a slide and exposed to the action of a very dilute solution of silver nitrate. Minute droplets of this mixture were then transferred to a clean slide and placed under the microscope. What were clear droplets became, on exposure to the sun, dark brown rings. The same thing occurs when any stain is evaporated: what was a droplet becomes on evaporation a ring with a faintly stained center. This is too common an observation to need mention. The rings produced by evaporating small drops of silver and lymph (or whatever the composition of the substance removed may be) bear a striking resemblance to the stomata produced in the regular manner, only of course they are larger. The discovery was accidentally made that, if the two substances were mixed in larger amounts and allowed to evaporate and then examined in glycerine, dots and rings appeared indistinguishable from the true stigmata and stomata. So striking are the pictures so produced that the statement seems warranted that stigmata and stomata may be produced independent of a serous membrane. This statement is equivalent to saying that the stigmata and stomata are the product of the action of a solution of silver nitrate upon lymph. The microscopic appearance of silver chloride bears a very strong resemblance to the product of silver and lymph, though the color is different. Dots corresponding to the stigmata may be seen and a few rings, but they are much less constant than in the silver and lymph product. It may be mentioned in passing that the granules seen in the tissues in argyra bear a very strong resemblance to these dots of silver chloride.

In order to test the silver chloride theory of the formation of the stigmata and stomata, the following experiment was performed: Five cc. of a sodium chloride solution was injected into the free peritoneal cavity of a mouse. After a few minutes a dilute solution of silver nitrate was injected through the same needle. After the silver solution had had time to mix thoroughly with the sodium chloride solution, the needle was withdrawn, the opening made by the needle ligated, and the animal given the liberty of the cage. Presumably the silver was precipitated as soon as it passed the point

of the needle, though absolute proof of this is difficult to produce, since the reaction takes place in a perfectly dark chamber, namely the free peritoneal cavity. Attempts were made to obtain an impregnation by first mixing the silver nitrate with the sodium chloride solutions and afterwards injecting. The results were negative. A trial was then made of the effect of different colored lights on these solutions made before injection. The results likewise were negative. The remarkable fact was noted that when the silver solution was preceded by a sodium chloride solution the lines appeared at once. When the silver solution was alone injected the lines did not appear until from one-half to three hours afterwards. In either case the appearance of the lines is the same. The difference was noted that when the silver solution was preceded by a sodium chloride solution there always occurred precipitates over the body of the cell, which is not the case when the silver is used alone. An attempt was made to change the reaction of the albuminous substance covering the peritoneum before injecting the silver solution. The means employed to accomplish this were to inject a solution of equal parts of hydrochloric acid (C. P.) and distilled water, followed after a few minutes by the silver solution. The lines appeared. The reader is allowed to judge for himself what effect an acid of this strength would have upon the peritoneal lymph. It was interesting to note that the endothelial cells stood this severe treatment with little injury. It was interesting, too, to note that when so treated the intercellular lines were very fine, with no broadenings whatever.

As an example of the striking figures produced by the action of the silver nitrate solution on an albumin solution figs. 1 and 2, pl. XIII, may be referred to. The specimen from which this drawing was taken was made by mixing a drop of ½ per cent of hydrochloric acid with a dilute albumin solution, and the mixture gently warmed and allowed to evaporate. The peculiar radiating arrangement of the rows or rings is due, no doubt, to the properties of the albumin solution.

What the exact composition of the product of silver and albumin is can not be stated. The fact that the fluids of the body contain 0.65 per cent of sodium chlorid, taken together with the well-known fact that the nitrate of silver in solution is more sensitive to chlorids in loose combination than to any other substance, has caused the

writer to accept as a working hypothesis the assumption that the substance is a chlorid of silver, perhaps holding an albuminous substance as an admixture. This assumption has to support it the following facts: (1) That no such substance as silver albuminate is known; (2) only when a chlorid-containing substance is present are they formed; (3) the sensitiveness of the silver nitrate in solution to chlorids in loose combination; (4) combinations of silver with nuclein are known, but nucleins are not contained in the substance in question.

This is but an hypothesis, it is true, and perhaps one of uncertain importance, but the fact remains that it is an unwarranted assumption to refer to any substance as silver albuminate. The ultimate solution of this problem may be left to those skilled in chemistry. Indeed, so far as the problems in the histology of the peritoneum are concerned, the exact composition of these substances is a matter of little importance.

But it is a problem of vastly greater importance to recognize them as chemical products, the product of the action of silver nitrate in solution upon a chlorid-containing substance. That the stigmata and stomata occur by no other method has already been stated, and the converse is equally true—that by all other methods, without a single exception, the endothelial cells are shown to contain no such openings.

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EXPLANATION OF PLATES [15]

Plate XII

Fig. 1. Mesentery of cat, showing a variety of stomata in the body of the cell. These structures differ from the intercellular stomata only in location.

Fig. 2. Mesentery of rabbit: *a*, numerous stigmata; *b*, stomata, one of them half stigma and half stoma, *c*, these structures here shown are not cells, as has been supposed, but are spurious products, the black center lying above, the circle below, the endothelial cells.

Fig 3. Mesentery of rabbit. A stoma between muscle cells below endothelial cells.

Fig. 4. Mesentery of frog showing a number of intercellular stigmata.

Fig. 5. Mesentery of rabbit showing stigmata on cross-section. They are located above the intercellular line.

Fig. 6. Mesentery of rabbit showing in the intercellular line a nucleus of a cell below the endothelial cells. Whether this nucleus belongs to the connective tissue cells, or to endothelial cells located below the regular layer, or to clasmatocytes, is uncertain.

Plate XIII

Fig. 1. Rings formed on a slide from a treatment of dilute albumin solution with silver nitrate solution. The rings in this striking figure are indistinguishable from those formed on serous membranes in the regular way.

Fig. 2. Peculiar tracings made by the same method used in fig. 1. This shows in a striking manner the variable figures the solutions named are capable of producing.

Fig. 3. Mesentery of young rabbit treated with silver solution after exudation had been excited with an irritant.

Fig. 4. Cross-section of specimen from which fig. 3 was taken. It shows that the heavy black precipitate shown in the preceding figure is located above the regular endothelial cells, and not between them, as has been generally believed from a study on the flat surface alone. The endothelial cells have been purposely torn from the membrana limitans for a short distance and left in position the remainder of the section. In the center of the figure a few fibers belonging to the basement membrane are still adherent to the cells.

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[15] All figures except figs. 1 and 2, pl. XIII, are redrawn from pencil sketches made with the aid of the camera lucida with Leitz Obj. 1/12, Oc. 4. The exceptions noted were drawn direct with Obj. 6, and Oc. 4.